SEX-DIVERGENT IMPACT OF DOCOSAHEXAENOIC ACID SUPPLEMENTATION ON THE LUNG OF POSTNATAL GROWTH RESTRICTED RATS

by

Claire Sorensen Bagley

A thesis submitted to the faculty of
The University of Utah
in partial fulfillment of the requirements for the degree of

Master of Science

Department of Nutrition and Integrative Physiology

The University of Utah

May 2018

The University of Utah Graduate School

STATEMENT OF THESIS APPROVAL

The thesis of **Claire Sorensen Bagley**

has been approved by the following supervisory committee members:

Lisa Joss-Moore	, Chair	**3/12/18** Date Approved
Julie Metos	, Member	**3/13/18** Date Approved
Jean Zancanella	, Member	**3/13/18** Date Approved

and by **Scott Summers** , Chair/Dean of

the Department/College/School of **Nutrition and Integrative Physiology**

and by David B. Kieda, Dean of The Graduate School.

ABSTRACT

Preterm infants are at increased risk for developing bronchopulmonary dysplasia (BPD). BPD is characterized by impaired lung development, with decreased lung compliance and increased lung tissue resistance. Decreased airway resistance is a related complication. Male infants typically experience worse outcomes of BPD than female infants and have a higher risk for acquiring the disease. BPD is induced by poor lung development, mechanical ventilation, and postnatal growth restriction (PGR) related to feeding complications. Poor feeding can result in limited consumption of specific nutrients essential to lung development, including docosahexaenoic acid (DHA). Decreased circulating DHA levels have been found in preterm infants who develop BPD. However, outcomes from clinical studies examining the effects of postnatal DHA supplementation on BPD outcomes are conflicting.

DHA is a ligand for the transcriptional regulator peroxisome proliferator-activated receptor gamma (PPARγ), a nuclear receptor transcription factor that contributes to lung development. The SET-domain containing histone methyltransferase (Setd8) is a transcriptional target of PPARγ in the lung, and serves as a useful downstream parameter for assessing PPARγ activity. We hypothesize that postnatal DHA supplementation will have a dose- and sex-dependent impact on lung function and structure, lung PPARγ and Setd8 protein, and Setd8 expression, and circulating fatty acids in the juvenile rat.

To test our hypothesis, we induced PGR in Sprague Dawley rat pups and observed the effect of high (0.1%), low (0.01%), and no DHA (0.0%) supplementation compared to age- and sex-matched controls. Lung function was measured through tissue compliance, tissue damping, and airway resistance. PPARγ and Setd8 mRNA transcript and protein levels were measured in lung homogenate. Sex-divergent outcomes were found in PPARγ protein, Setd8 protein, and tissue damping. DHA was found to ameliorate the impact of PGR in all functional tests, with females showing greater sensitivity to DHA in restoration of compliance.

In conclusion, DHA causes sex- and dose-dependent changes in lung function and lung PPARγ levels in PGR rat pups. We speculate that sex-divergent responses to DHA reflect sex-divergent metabolism of DHA. We further speculate that sex-divergent effects of PGR on lung mechanics may reflect differences in the structural organization and composition of the lung.

TABLE OF CONTENTS

LIST OF FIGURES

Figures

ACKNOWLEDGMENTS

I would first like to acknowledge my committee chair, Lisa Joss-Moore. I feel so fortunate to have been a member of the Joss-Moore lab, and have been taught well by the experience. Lisa's intelligent, compassionate mentoring has shaped both my academic and personal development.

I would also like to thank my committee members, Dr. Julie Metos and Jean Zancanella, for their support throughout this process. I have appreciated their instruction and friendship over the course of the Coordinated Master's Program.

To every success at the bench I attribute the help of Mariana Macedo de Almeida and Haimei Wang, invaluable teachers and companions in the lab. I would also like to give special thanks to Amy Loverin for her camaraderie and moral support over the course of this research journey.

Lastly, I wish to thank my wonderful parents, siblings, grandparents, and my husband, Colin, for their interest, support, love, and encouragement.

INTRODUCTION

Bronchopulmonary Dysplasia and Postnatal Growth Restriction

Bronchopulmonary dysplasia (BPD) affects 10,000 preterm infants in the United States each year.[1] BPD, the chronic lung disease of early infancy, results in high mortality and increased risk of comorbidities for premature infants. Survivors often suffer long-term consequences, including deficits in lung health over the lifetime.

Preterm birth influences the development of BPD through several mechanisms. Weeks 28-32 of gestation are a critical period for lung development.[2] Consequently, the lungs of preterm infants, typically born at 28 to 32 weeks gestational age, are underdeveloped. Furthermore, preterm infants usually require respiratory support. While necessary for survival, respiratory support exposes the infants to a hyperoxic breathing environment, which induces further manifestation of BPD.[3] Mechanical ventilation, one common form of respiratory support, has the additional drawback of interfering with preterm infant feedings, which in turn influences the development of BPD.[4] Male infants typically experience worse outcomes of BPD than female infants and have a higher risk for acquiring the disease.[5]

Feeding complications related to mechanical ventilation, or other comorbidities of preterm birth, put preterm infants at greater risk for postnatal growth restriction (PGR). PGR is generally defined as "…a weight less than the 10th percentile for corrected gestational age at the time of hospital discharge."[6] The sedation required for mechanical

feeding often contributes to severe caloric deficits in preterm infants, who are also prone to feeding intolerance and critical illness, further impeding their ability to consume sufficient nutrients. These factors combine to induce PGR and augment the presentation of BPD.[6,7]

Sex-divergent Effects of PGR on Lung Function

BPD augmented by PGR impacts several aspects of lung function. The disease is characterized by impaired lung development, with decreased lung compliance and increased lung tissue resistance.[8] Decreased airway resistance is a related complication.[9] Preliminary work by our lab indicates that these conditions display sex-divergent phenotypes, with males being at higher risk of increased lung tissue damping and females being at a higher risk for increased airway resistance, with both sexes showing similar risk for decreased lung compliance.

DHA and Lung Function

Docosahexaenoic acid (DHA), an Omega-3 (ω-3) long chain polyunsaturated fatty acid (PUFA), has been shown to affect fetal lung development.[10,11,12] Altered levels of circulating fatty acids, including decreased DHA, have been found in preterm infants who develop BPD.[13] Significantly, DHA is a ligand for the transcriptional regulator peroxisome proliferator-activated receptor gamma (PPARγ).[14] Nutrient activation by ligands such as DHA is essential to lung development. However, outcomes from clinical studies examining the effects of postnatal DHA supplementation on BPD outcomes are

conflicting. **As a result, consensus on dose and sex effects of DHA supplementation in human PGR infants is lacking.**

As previously stated, in rats, PGR causes sex-divergent deficits in lung function. The effects of PGR on circulating DHA, and the impact of postnatal supplementation with DHA, are also sex-divergent. Previous work by our lab shows that PGR decreases circulating DHA in male rats, but not female rats. These differences present a potential avenue for refining the administration of DHA and maximizing its benefit to the preterm infant with BPD.

PPARγ and Lung Function

PPARγ is a member of the PPAR family of nuclear receptors. As a group, they are involved in many important regulatory pathways, including fat metabolism, cell differentiation and proliferation, and inflammation.[15] There are two isoforms of the PPARγ gene, PPARγ1 and PPARγ2, produced with different transcriptional starting points. Both isoforms are expressed in the lung and are required for lung development.[12] PPARγ ligands, including DHA, activate both isoforms. DHA has displayed a higher binding affinity for PPARγ compared to other long chain fatty acids.[16] This knowledge makes PPARγ a prime target of study for the treatment of infant lung disease, specifically, BPD.

As with the development of BPD and the impact of DHA supplementation, PPARγ expression in the lung differs by sex. Previous work by our lab demonstrated that PPARγ expression is higher in male rat pups without PGR than female rat pups without PGR, whereas PGR decreases that expression in males but not in females.[17]

Setd8

The SET-domain containing histone methyltransferase (Setd8) functions as an epigenetic modifier and as such can affect gene expression. It places a monomethyl (Me) group on lysine (K) 20 of Histone (H) 4, and is one transcriptional target of PPARγ.[18] PPARγ is, in turn, a target of Setd8 activity. In our rat model of intrauterine growth restriction, we demonstrated that intrauterine growth restriction (IUGR) decreased protein levels of lung PPARγ and Setd8 independent of gender, with outcomes normalized by maternal DHA.[12] The same study indicated that levels of the Setd8-dependent histone modification, H4K20Me, were reduced on the PPARγ gene in rat pups with intrauterine growth restriction, representing a possible feedback loop through epigenetic modification. Because of this relationship, Setd8 serves as a useful downstream parameter for assessing PPARγ activity.

Hypothesis

We hypothesize that postnatal DHA supplementation will have a dose- and sex-dependent impact on lung function and lung PPARγ and Setd8 expression in the juvenile rat.

METHODS

Animal Model

PGR was induced in Sprague Dawley rat pups using variation in litter size.[1] Newborn rat pups were cross-fostered to rat dams with litter sizes of 16 (PGR) or 8 (control). Each litter was randomized to receive diets supplemented with DHA at 0.0%, 0.01%, and 0.1%. Rat pup weight was measured every other day from day of life 0 (d0) to d21. At d21, control and PGR rat offspring were euthanized using a sodium pentobarbital overdose. At d21, serum and lung tissue were collected and immediately flash frozen in liquid nitrogen.

DHA Supplementation

Pregnant rats were fed either standard rodent chow or a custom diet containing 0.01% or 0.1% DHA. The custom diets were based on Harlen Teklad 8640 standard rodent diet (TD.8640, Harlan-Teklad, WI), substituting 0.01% and 0.1% DHA for 0.01% and 0.1% of the standard soybean oil, respectively. All other macronutrient content was maintained compared to standard rodent chow (21.8% protein, 40.8% carbohydrate, and 5.4% fat, with a caloric density of 3 Kcal/g). The pregnant rats were fed one of the three diets from E13 until term. Food intake was independent of diet.

Measurements of Lung Mechanics

The mechanical properties of the PGR and control rat lungs on regular and both DHA diets were measured using the FlexiVent system (Scireq, Canada). Rats were anesthetized, paralyzed, and ventilated.

We measured static lung compliance, as well as tissue damping and airway resistance.

mRNA and Protein Analysis

PPARγ and Setd8 mRNA transcript levels were measured in lung homogenate from PGR and control rat lungs on regular and both DHA diets using real-time reverse transcriptase PCR, with the following Assay on Demand: PPARγ Rn01492274_m1 (Thermo Fischer Scientific), and Setd8 Rn01477383_g1 (Thermo Fisher Scientific). PPARγ and Setd8 protein abundance were measured using Western blotting. The primary antibody used was PPARγ (B5) (Santa Cruz, TX). GAPDH was used as a housekeeping gene for RT PCR and Western blotting.

Statistical Analysis

PGR rat pups were compared to sex-matched control pups. Male and female rats were considered separate groups. Sample size was n=4-20 per group. GraphPad Prism was used for statistical analysis. One-way ANOVA with post hoc test was used to detect differences between groups. Statistical significance was defined as $p<0.05$.

RESULTS

Growth of Rat Pups

In both female and male rat pups, body weight of PGR pups was significantly less than sex-matched control by postnatal day 5, and continued to be significantly less through postnatal day 21, with the magnitude of growth restriction comparable in both male and female rat pups. Body weights of PGR rat pups are not affected by either DHA diet (see Figure 1).

Lung Mechanics

In male rats, PGR decreased lung compliance. Lung compliance remained significantly less than control levels with the 0.01% diet, but was not significantly different on the 0.1% diet (see Figure 2). In female rats, PGR also decreased lung compliance. However, lung compliance was restored to control levels by both the 0.01% and 0.1% DHA diet (see Figure 3).

In male rats, PGR increased tissue damping. No statistical difference in tissue damping was found between the control on regular diet and the PGR pups on either DHA diet (see Figure 4). In female rats, neither PGR nor DHA supplementation affected tissue damping (see Figure 5).

In male rats, PGR increased airway resistance, with restoration by both DHA diets (see Figure 6). Likewise, airway resistance was increased by PGR in female rats, and

normalized by both DHA doses (see Figure 7).

PPARγ Results

Both PPARγ mRNA variants were measured as both have been shown to play a role in lung development. In males, PGR decreased PPARγ1 mRNA levels. Levels were further decreased by supplementation with both the 0.01% DHA diet and the 0.1% DHA diet (see Figure 8). PPARγ1 mRNA levels were not affected significantly by PGR in females, but similar to males were reduced by both DHA diets (see Figure 9). Similarly to PPARγ1, PGR reduced PPARγ2 mRNA levels in males, with further reductions by DHA supplementation (see Figure 10). PPARγ2 mRNA levels in females likewise followed the same pattern as seen in PPARγ1, with no reduction evident from PGR, but with depressed levels by DHA supplementation (see Figure 11).

PPARγ protein levels were reduced by PGR in males, but not in females. The low DHA diet did not change protein levels significantly from PGR pups on a regular diet, but restoration to normal levels was seen with the high dose of DHA. No significant changes in PPARγ protein levels were seen in females from any of the interventions (see Figures 12 and 13).

Setd8 Results

Changes in Setd8 mRNA levels followed the same pattern in males as in females. PGR increased Setd8 levels, while both doses of DHA restored levels to normal, with no significant difference between doses (see Figures 14 and 15). In male pups, Setd8 protein levels were increased significantly by PGR. Both doses of DHA supplementation further

increased protein levels, with no significant difference between doses (see Figure 16). In female pups, no significant differences were observed between the control and PGR infants on regular or DHA diets (see Figure 17).

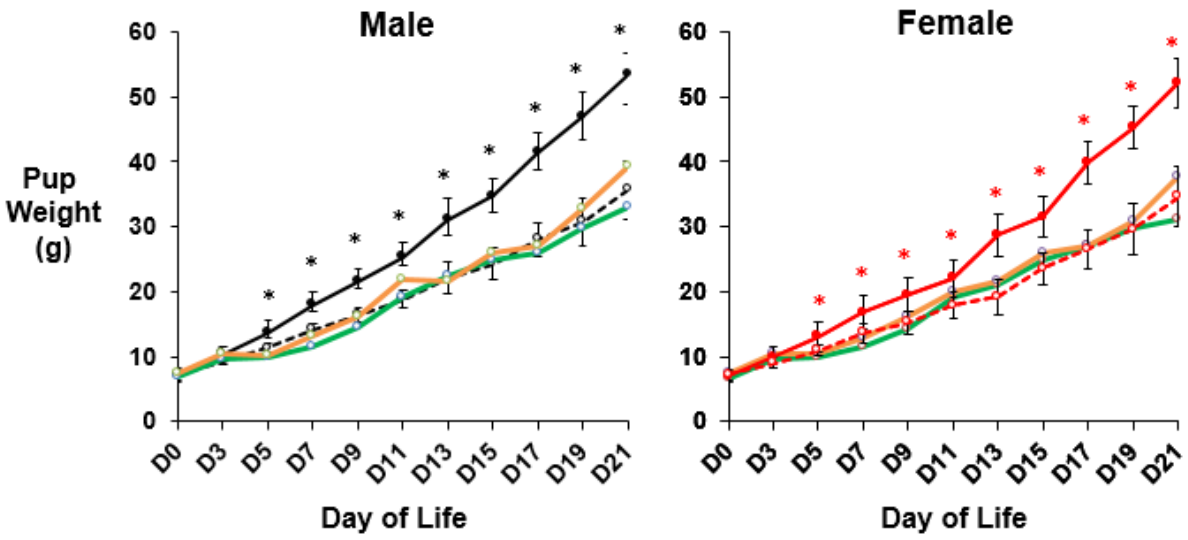

Figure 1. Growth curves of control rat pups on regular diet and PGR rat pups on regular, 0.01%, and 0.1% DHA diet

Growth of PGR rat pups was significantly less than control among both males and females and was unaffected by DHA supplementation (n=9-20; *p<0.05). Control rat pups are represented with the solid black and red lines, and PGR rat pups with the dashed black and red lines. The green and orange lines on each growth curve show the PGR rats receiving 0.01% and 0.1% DHA diet, respectively.

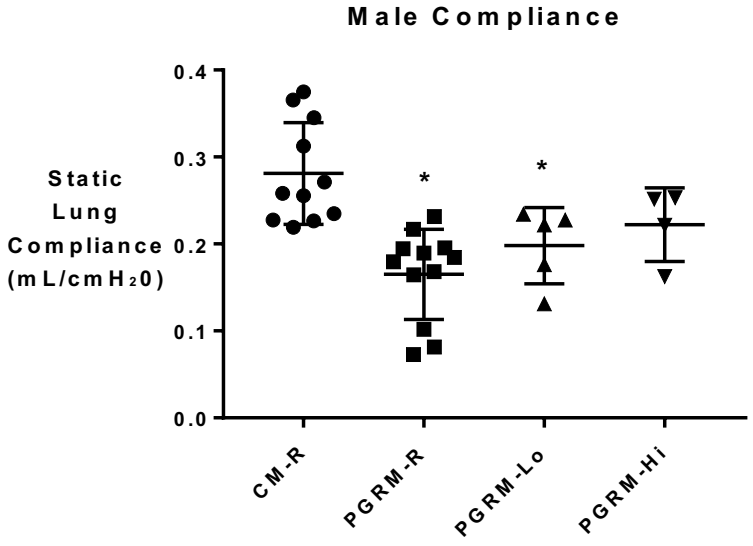

Figure 2. Static lung compliance of male control rat pups on regular diet and male PGR rat pups on regular, 0.01%, and 0.1% DHA diet
Lung compliance of male rat pups was significantly reduced by PGR and restored to normal levels by a high dose of DHA supplementation (n=5-12; *p<0.05).

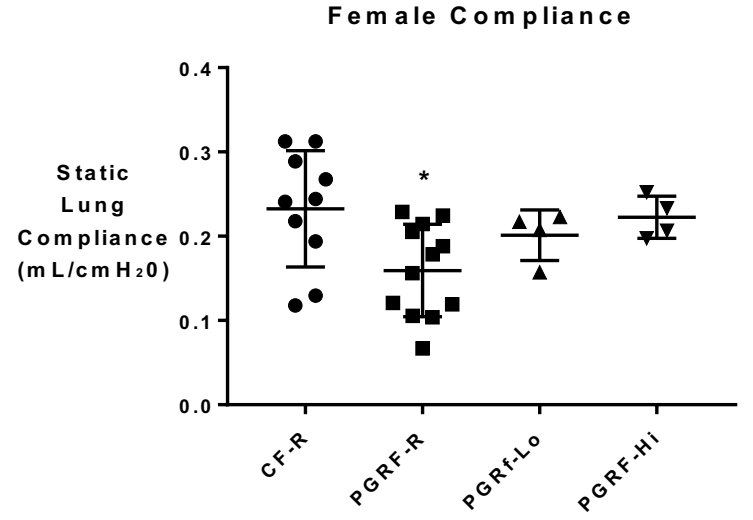

Figure 3. Static lung compliance of female control rat pups on regular diet and female PGR rat pups on regular, 0.01%, and 0.1% DHA diet
Lung compliance of female rat pups was significantly reduced by PGR and restored to normal levels by a low dose of DHA supplementation (n=4-12; *p<0.05).

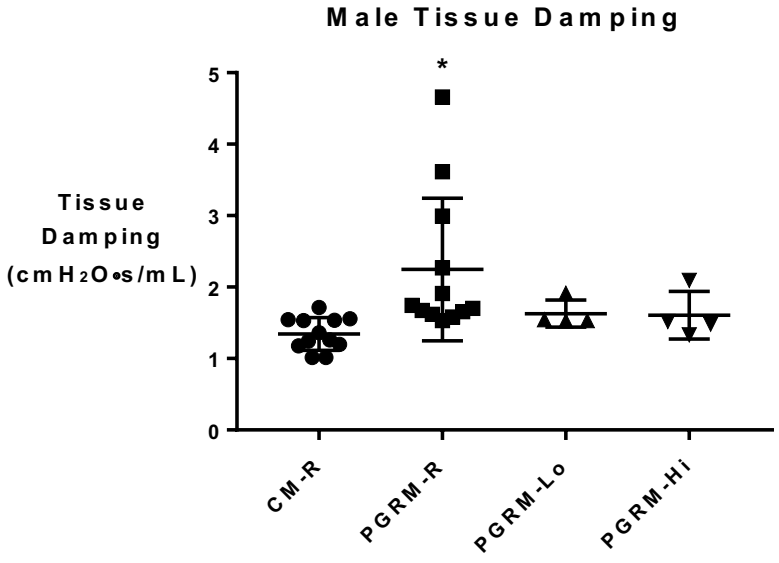

Figure 4. Tissue damping of male control rat pups on regular diet and male PGR rat pups on regular, 0.01%, and 0.1% DHA diet
Tissue damping of male rat pups was significantly increased by PGR and restored to normal levels by a low dose of DHA supplementation (n=4-12; *p<0.05).

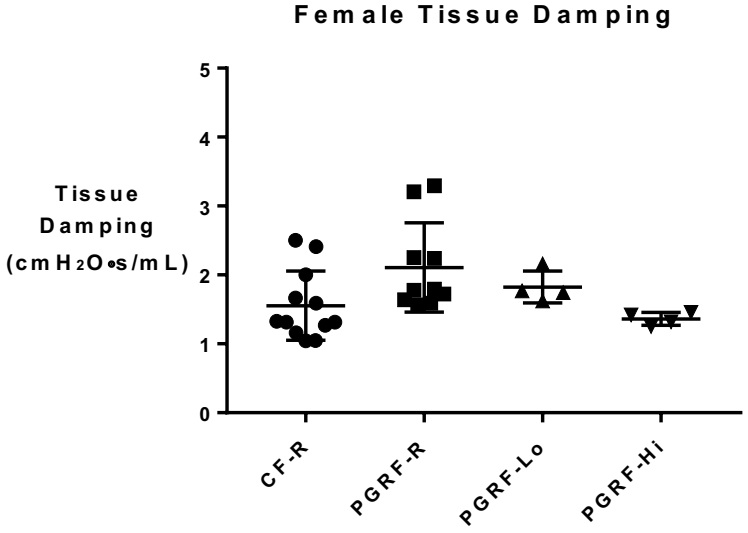

Figure 5. Tissue damping of female control rat pups on regular diet and female PGR rat pups on regular, 0.01%, and 0.1% DHA diet
Tissue damping of female rat pups was not significantly by either PGR or DHA supplementation (n=4-12).

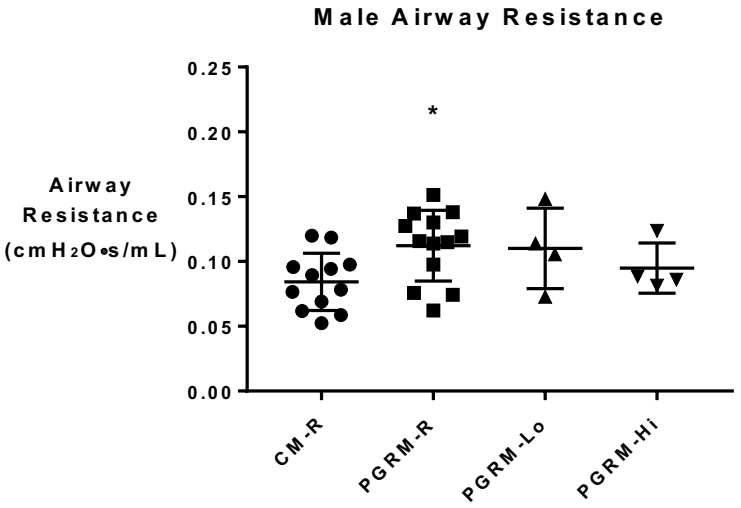

Figure 6. Airway resistance of male control rat pups on regular diet and male PGR rat pups on regular, 0.01%, and 0.1% DHA diet
Airway resistance of male rat pups was increased by PGR and restored to normal levels by a low dose of DHA supplementation (n=4-13; *p<0.05).

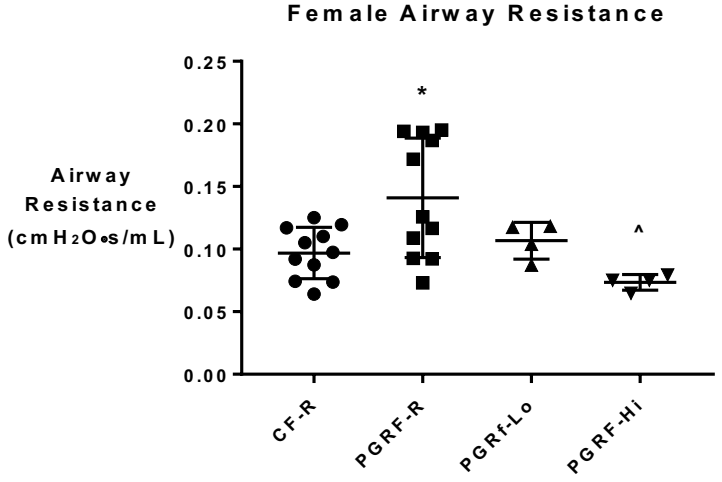

Figure 7. Airway resistance of female control rat pups on regular diet and female PGR rat pups on regular, 0.01%, and 0.1% DHA diet
Airway resistance of female rat pups was increased by PGR and restored to normal levels by a low dose of DHA supplementation (n=4-11; *p<0.05 compared to control on regular diet; ^p<0.05 compared to PGR on regular diet).

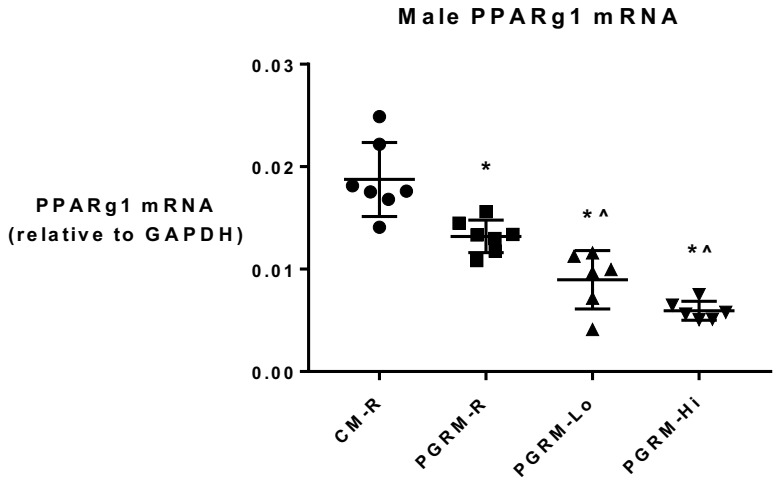

Figure 8. PPARγ1 mRNA transcript levels in male control rat pups on regular diet and male PGR rat pups on regular, 0.01%, and 0.1% DHA diet

PPARγ1 mRNA transcript levels in male rat pups were significantly reduced by PGR, and again by a low dose of DHA supplementation (n=6-7; *p<0.05 compared to control on regular diet; ^p<0.05 compared to PGR on regular diet).

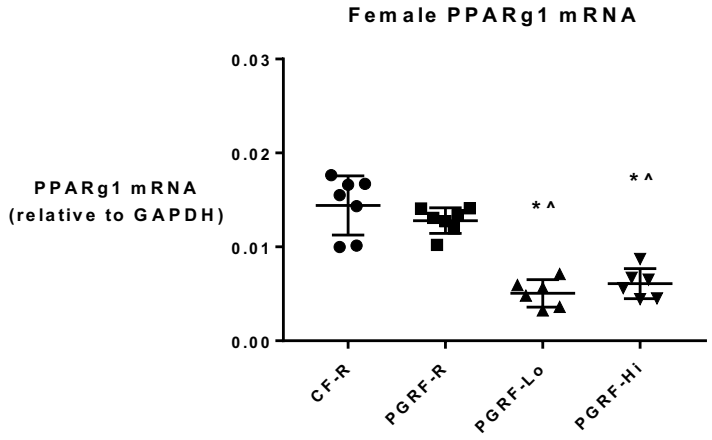

Figure 9. PPARγ1 mRNA transcript levels in female control rat pups on regular diet and female PGR rat pups on regular, 0.01%, and 0.1% DHA diet

PPARγ1 mRNA transcript levels in female rat pups were not changed significantly by PGR, and significantly reduced by a low dose of DHA supplementation (n=6-7; *p<0.05 compared to control on regular diet; ^p<0.05 compared to PGR on regular diet).

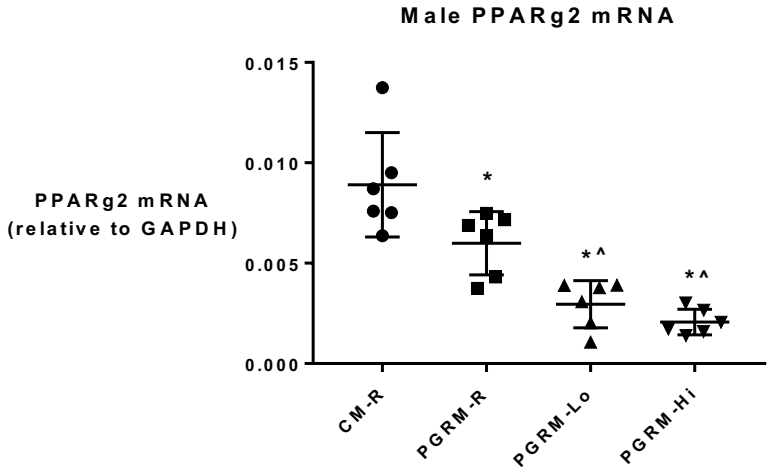

Figure 10. PPARγ2 mRNA transcript levels in male control rat pups on regular diet and male PGR rat pups on regular, 0.01%, and 0.1% DHA diet

PPARγ2 mRNA transcript levels in male rat pups were significantly reduced by PGR, and again by a low dose of DHA supplementation (n=6; *p<0.05 compared to control on regular diet; ^p<0.05 compared to PGR on regular diet).

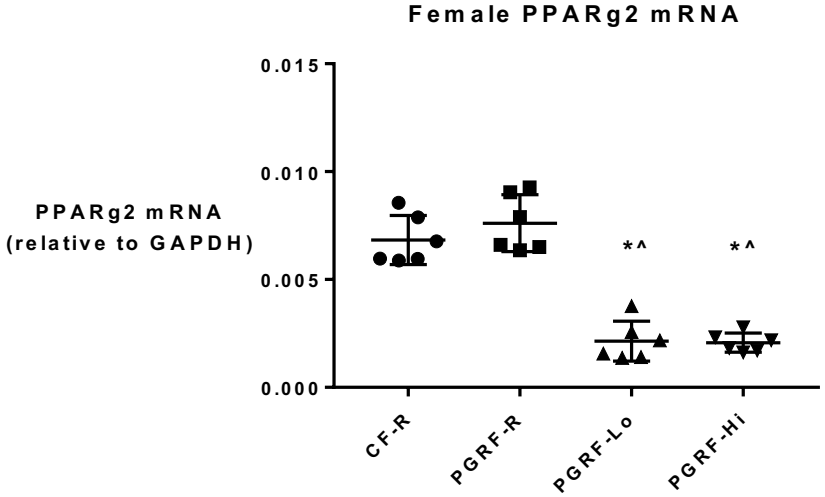

Figure 11. PPARγ2 mRNA transcript levels in female control rat pups on regular diet and female PGR rat pups on regular, 0.01%, and 0.1% DHA diet

PPARγ2 mRNA transcript levels in female rat pups were not changed significantly by PGR, and significantly reduced by a low dose of DHA supplementation (n=5-6; *p<0.05 compared to control on regular diet; ^p<0.05 compared to PGR on regular diet).

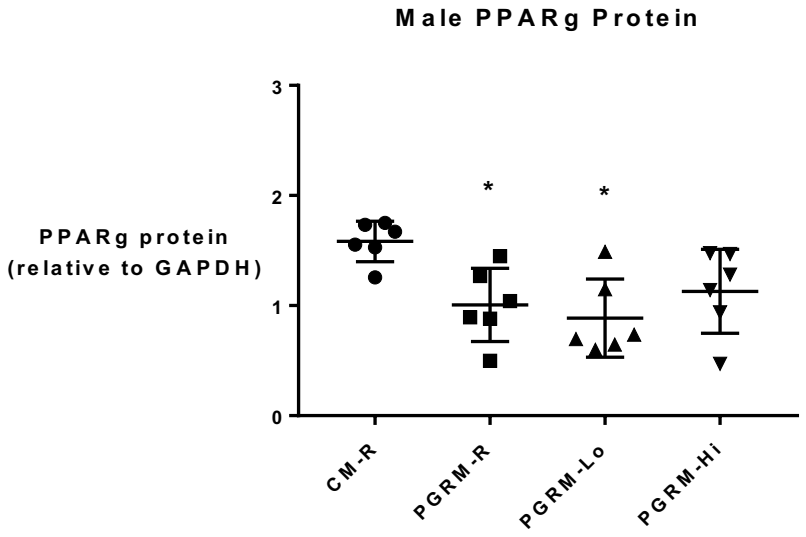

Figure 12. PPARγ protein levels in male control rat pups on regular diet and male PGR rat pups on regular, 0.01%, and 0.1% DHA diet

PPARγ protein levels in male rat pups were significantly reduced by PGR and restored to normal levels by a high dose of DHA supplementation (n=6; *p<0.05).

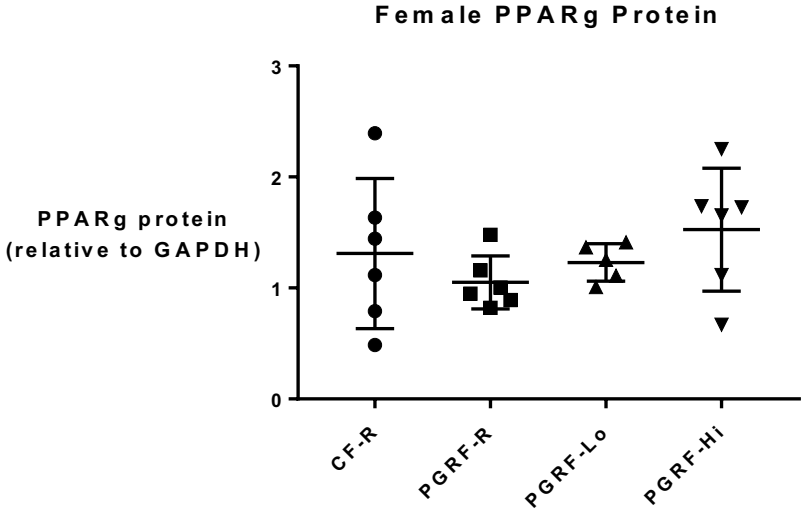

Figure 13. PPARγ protein levels in female control rat pups on regular diet and female PGR rat pups on regular, 0.01%, and 0.1% DHA diet

PPARγ protein levels in female rat pups were not significantly altered either by PGR or by DHA supplementation (n=5-6).

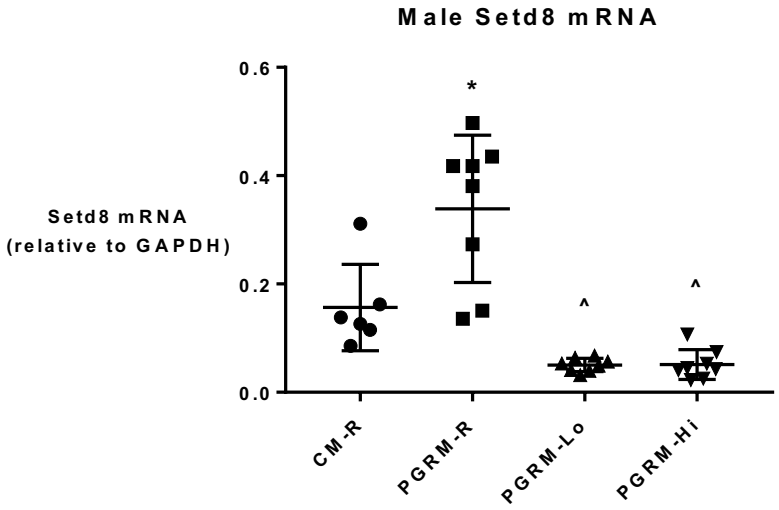

Figure 14. Setd8 mRNA transcript levels in male control rat pups on regular diet and male PGR rat pups on regular, 0.01%, and 0.1% DHA diet
Setd8 mRNA transcript levels in male rat pups were significantly increased by PGR and restored to normal levels by a low dose of DHA supplementation (n=6-8; *p<0.05 compared to control on regular diet; ^p<0.05 compared to PGR on regular diet).

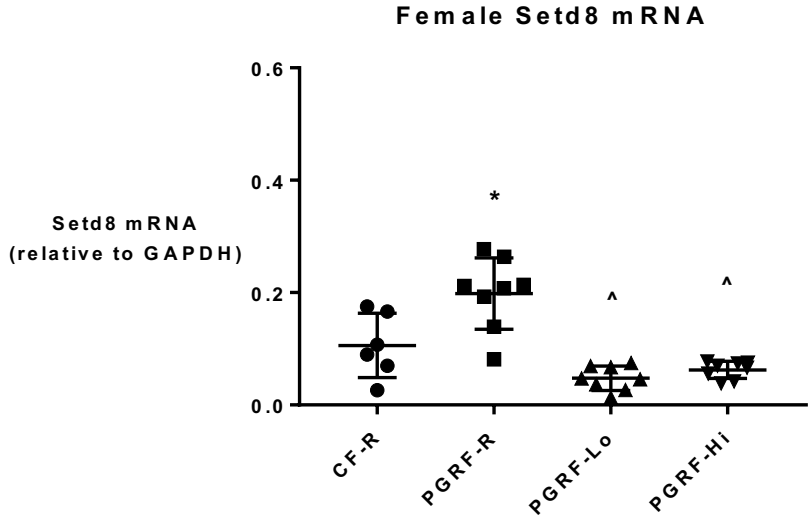

Figure 15. Setd8 mRNA transcript levels in female control rat pups on regular diet and female PGR rat pups on regular, 0.01%, and 0.1% DHA diet
Setd8 mRNA transcript levels in female rat pups were significantly increased by PGR and restored to normal levels by a low dose of DHA supplementation (n=6-8; *p<0.05 compared to control on regular diet; ^p<0.05 compared to PGR on regular diet).

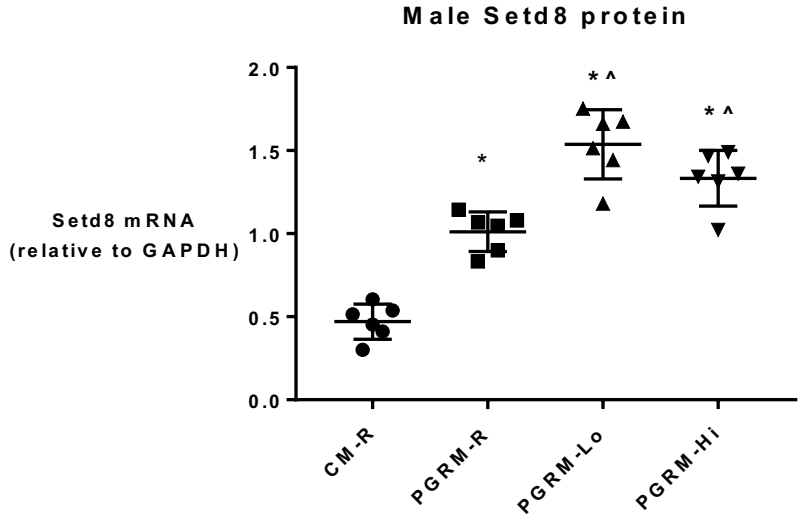

Figure 16. Setd8 protein levels in male control rat pups on regular diet and male PGR rat pups on regular, 0.01%, and 0.1% DHA diet

Setd8 protein levels in male rat pups were significantly increased by PGR and again by a low dose of DHA supplementation (n=6; *p<0.05 compared to control on regular diet; ^p<0.05 compared to PGR on regular diet).

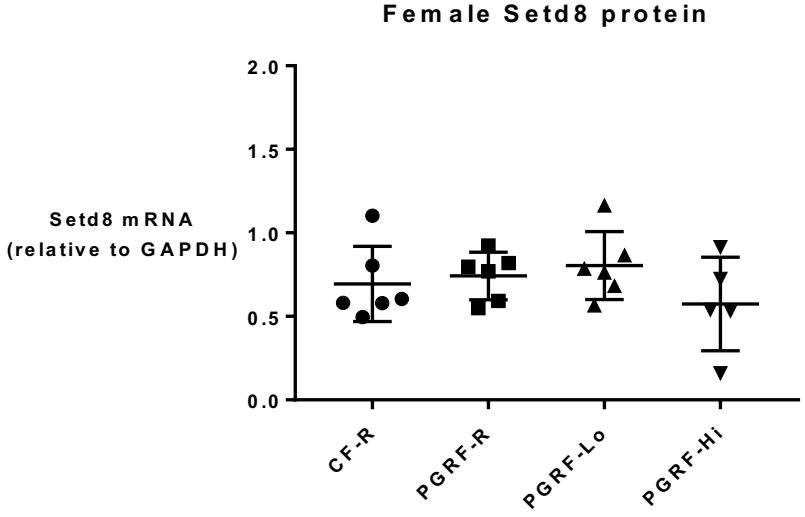

Figure 17. Setd8 protein levels in female control rat pups on regular diet and female PGR rat pups on regular, 0.01%, and 0.1% DHA diet

Setd8 protein levels in female rat pups were not significantly altered by PGR or DHA supplementation (n=5-6).

DISCUSSION

As a treatment for BPD, DHA has shown clinical promise, and its relevance is widely accepted in the field of neonatal pulmonology. The third trimester is a time of significant DHA accrual in fetal tissue, and because of this, supplemental DHA may play an especially beneficial role for preterm infants.[19] The idea that DHA may be important for lung development began with the observation that circulating DHA levels were decreased in preterm infants who develop BPD.[13]

One possible mechanism for the impact of DHA on lung development is its interaction with the nuclear transcription factor PPARγ. When activated by a ligand, this nuclear receptor protein plays many important roles in the lung, including maintaining lipid homeostasis, mediating intercellular communication, and regulating epigenetic enzymes, including Setd8.[12,15] PPARγ is well known to influence BPD outcomes through mediation of alveolar homeostasis, and treatment with PPARγ agonists appears to effectively ameliorate aspects of BPD pathogenesis.[20] DHA has been shown to activate and possess a high binding affinity for both isoforms of PPARγ.[16,21] The potential benefit of DHA to infant lung development may work, at least in part, through this mechanism.

Supplemental DHA in preterm infants has been shown to improve lung architecture and responses to respiratory challenges.[10] On the other hand, the same research suggests longer and more frequent apneas consequent to ω-3 supplementation. Furthermore, a recent study showed that enteral DHA supplementation did not result in a

lower risk of BPD than a control emulsion among preterm infants born before 29 weeks of gestation and may have resulted in a greater risk.[22]

These latter findings call into question the appropriateness of supplemental DHA in acute care for preterm infants. However, in that study, dosage was standardized at 60 mg/kg/day and no distinction based on sex was made in the analysis. Previous work by our lab shows that circulating essential fatty acids, including DHA, are altered by PGR in a sex-divergent fashion, suggesting that response to DHA supplementation may vary by sex.[17] Our current study aimed to interrogate the role of both sex and dose in outcomes for supplemental DHA. We found that DHA supplementation restored all functional outcomes to control levels in both male and female PGR pups, lending further support to its potential as an intervention for BPD. We hypothesized that the impact of DHA supplementation would differ between sexes, and a sex-divergent response was observed in PPARγ protein, Setd8 protein, lung tissue compliance, and tissue damping.

We likewise found that DHA had the anticipated impact on PPARγ protein levels. BPD affects male infants more severely than it does female infants, and PGR magnifies those symptoms. Given this background, the reduction in PPARγ in male rat pups consequent to PGR, and the lack thereof in female rat pups, is not surprising. The restoration of PPARγ protein levels by DHA further reinforces its potential benefit to infants with BPD.

In correspondence with the trend seen in PPARγ protein, we observed that PGR decreases PPARγ mRNA in males but not females. However, the decrease in PPARγ mRNA with DHA contrasts sharply with the restoration seen in PPARγ protein and occurred in both sexes. This is suggestive of tight regulatory mechanisms. By activating

PPARγ, DHA may play a role in increasing the efficiency of protein that has already been translated, thereby diminishing the need to transcribe more copies of the gene and causing it to be downregulated. This notion is reinforced by the restoration of PPARγ protein levels in males despite the continuing decrease in mRNA levels, suggesting that the restorative mechanism is not increased transcription, but rather preservation of the protein once it has been transcribed.

Setd8 was chosen as a molecular marker for PPARγ activity in this study because it has already been shown to be a target of PPARγ in the lung.[18] Activated PPARγ protein, functioning in its role as a transcription factor, binds to a PPAR response element – a sequence of nucleotides in the target gene recognized by PPARγ – and initiates transcription of Setd8. Setd8, in turn, places a methyl group on histone proteins associated with other genes, among them PPARγ, thereby altering their expression. Through this means, PPARγ can mediate the epigenetic environment of the cell.

Based on our knowledge of this pathway, we would expect that as PPARγ protein decreased in males in response to PGR, Setd8 mRNA would also be decreased, and as PPARγ protein remained constant in females, so would Setd8 mRNA. Contrary to our hypothesis, however, Setd8 mRNA increased in both males and females, and its response to DHA supplementation bears no association with that of PPARγ protein. It is not clear to us at this time why Setd8 did not show a direct response to PPARγ. Compared to our work in IUGR, which showed an increase in Setd8 mRNA corresponding with PPARγ, this opposite effect may reflect different compensatory mechanisms at work in utero versus ex utero. In utero, the fetus still has access to maternal nutrient stores and other forms of support. Once outside, the offspring as an organism is on its own, and may

compensate by downregulating or upregulating different pathways than it would with full access to maternal resources. Based on the significant changes in Setd8 mRNA in response to supplementation, however, we can surmise that DHA may influence Setd8 transcription through a means other than PPARγ.

The functional outcomes tell a similar story. Despite their congruence with the field, our PPARγ-related findings do not correspond with all functional outcomes measured in this study and have implications for the mechanistic involvement of PPARγ. Since airway resistance was increased by PGR in both sexes and restored in both by a low dose of DHA, while PPARγ was significantly reduced only in males, we may assume that PPARγ is not the mechanism behind the development of this phenotype. We speculate that airway resistance may be driven by inflammatory mediators, independent of PPARγ, which respond to DHA.

Similar to airway resistance, lung compliance was altered in both sexes, and restored by supplemental DHA. Here again, it seems unlikely that PPARγ was part of either the pathogenic or restorative pathways. Nevertheless, the results for lung compliance support our hypothesis that supplemental DHA would have sex-divergent outcomes. While restoration was seen in both sexes, in females, restoration was seen at a low dose, whereas males did not return to normal until the high dose, suggesting a greater sensitivity to DHA among female rat pups. This reinforces the notion that dosage considerations should be made when providing DHA supplementation in acute care settings.

Of the three functional outcomes measured, tissue damping is the only one to suggest possible influence by PPARγ. The alteration of tissue damping by PGR in males

but not females and its restoration by DHA is inversely associated with the trend in PPARγ protein levels, suggesting that PPARγ may be involved in creating the normal phenotype. While normalcy in PPARγ protein levels lagged behind restoration in tissue damping, this does not rule out the possibility that PPARγ plays a mechanistic role in the pathway if less than normal levels are required for restoration.

If PPARγ is involved in the increase in tissue damping, it may be acting through alterations in elastin deposition. Tissue damping reflects the energy dissipation in the alveoli, or what is known as tissue viscoelasticity. Semireflective of resistance to pressure, tissue damping also incorporates the speed at which the tissue will expand under a given pressure. This property is most likely related to the structural composition of the tissue, including the arrangement of elastin. Elastin, a component of alveolar walls, forms fiber-like structures through a process known as crosslinking. PPARγ has been shown to alter elastin deposition in the neonatal rat lung.[23] Additionally, work by our lab shows that the expression of elastin in the developing rat lung is altered by IUGR.[24] Other studies have found that alternative splicing of elastin can affect elastin crosslinking and modify disease severity.[25] Changes in alternative splicing and elastin crosslinking and deposition may be a factor in increasing tissue damping. We speculate that PPARγ may be involved in the process of alternative splicing and thereby modify the level of tissue damping. Future studies will involve measurement of elastin alternative splicing and assessment of elastin deposition and crosslinking and the influence of supplemental DHA thereon, in combination with a PPARγ inhibitor to identify or rule out its involvement.

There are limitations to this study. There are many factors that could shape the impact of DHA supplementation that were not assessed in this study, including the

developmental window of administration. While we speculate that DHA is operating through PPARγ in some cases and in others not, this supposition is based on association. Although DHA is a known activator of PPARγ, it is also known to impact macrophage polymerization and to influence inflammation as a precursor to eicosanoids and could potentially shape phenotype through these mechanisms.[26] To verify the pathway, future tests would involve administration of a PPARγ antagonist and observing whether or not the results remained intact.

In conclusion, DHA causes sex- and dose-dependent changes in lung function and lung PPARγ levels in PGR rat pups. We speculate that sex-divergent responses to DHA reflect sex-divergent metabolism of DHA. We further speculate that sex-divergent effects of PGR on lung mechanics may reflect differences in the structural organization and composition of the lung.

This study sheds light on the importance of dose and sex considerations in postnatal DHA supplementation. While much work has been done to investigate the relationship of DHA supplementation on the mechanisms of preterm infant lung development and the mechanistic role of PPARγ, work examining the divergent outcomes in males and females is limited. Identifying specific responses between the sexes furthers the development of more appropriate dosage recommendations for differing male and female physiologies. Though many beneficial outcomes for infants with BPD have been reported with DHA supplementation, other reports suggest a potentially harmful effect in some cases. Sex-adjusted dosages may be the key to maximizing the benefit of DHA supplementation for this population while avoiding any potential harm.

REFERENCES

1. Wedgwood S, Warford C, Agvateesiri SC, et al. Postnatal growth restriction augments oxygen-induced pulmonary hypertension in a neonatal rat model of bronchopulmonary dysplasia. *Pediatr Res.* 2016; (August). doi:10.1038/pr.2016.164

2. Copland I, Post M. Lung development and fetal lung growth. *Paediatr Respir Rev.* 2004;5(SUPPL. A). doi:10.1016/S1526-0542(04)90049-8.

3. Patel SC, Chen T, Sun M, Ramchandran R, Raj U. MicroRNA-133 mediated angiogenesis is suppressed in hyperoxia-induced bronchopulmonary dysplasia. *J Am Coll Cardiol.* 2017; (March). doi:10.1016/S0735-1097(17)35427-X

4. Joss-Moore LA, Hagen-Lillevik SJ, Yost C, et al. Alveolar formation is dysregulated by restricted nutrition but not excess sedation in preterm lambs managed by non-invasive support. *Pediatr Res.* 2016;(May):1-10. doi:10.1038/pr.2016.143

5. Joss-Moore LA, Albertine KH, Lane RH. Epigenetics and the developmental origins of lung disease. *Mol Genet Metab.* 2011;104(1-2):61-66. doi:10.1038/pr.2016.143

6. Ruth VA. Extrauterine growth restriction: a review of the literature. *Neonatal Netw.* 2008;27(3):177-184.

7. Biniwale MA, Ehrenkranz RA. The role of nutrition in the prevention and management of bronchopulmonary dysplasia. *Semin Perinatol.* 2006;30(4):200-208. doi:10.1053/j.semperi.2006.05.007.

8. Lui K, Lloyd J, Ang E, Rynn M, Gupta JM. Early changes in respiratory compliance and resistance during the development of bronchopulmonary dysplasia in the era of surfactant therapy. *Pediatr Pulmonol*. 2000;30(4):282-290. doi:10.1002/1099-0496(200010)30:4<282::AID-PPUL2>3.0.CO;2-D

9. Kao LC, Warburton D, Platzker ACG, Keens TG. Effect of isoproterenol inhalation on airway resistance in chronic bronchopulmonary dysplasia. *Pediatrics*. 1984;73(4):509-514

10. Tenorio-Lopes L, Baldy C, Jochmans-Lemoine A, et al. Consequences of maternal omega-3 polyunsaturated fatty acid supplementation on respiratory function in rat pups. *J Physiol.* 2016;(418):163-168. doi:10.1113/JP273471.

11. Swanson D, Block R, Mousa SA. Omega-3 fatty acids EPA and DHA: health benefits throughout life. *Adv Nutr An.* 2012:1-7. doi:10.3945/an.111.000893.Omega-3.

12. Joss-Moore LA, Wang Y, Baack ML, et al. IUGR decreases PPARγ and SETD8 expression in neonatal rat lung and these effects are ameliorated by maternal DHA supplementation. *Early Hum Dev.* 2011;86(12):785-791. doi:10.1016/j.earlhumdev.2010.08.026.IUGR.

13. Martin CR, Dasilva DA, Cluette-Brown JE, et al. Decreased postnatal docosahexaenoic and arachidonic acid blood levels in premature infants are associated with neonatal morbidities. *J Pediatr Pediatr Med*. 2011;159(5). doi:10.1016/j.jpeds.2011.04.039.

14. Krey G, Braissant O, L'Horset F, et al. Fatty acids, eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-activated receptors by coactivator-dependent receptor ligand assay. *Mol Endocrinol.* 1997:11(6):779-791. doi:10.1210/mend.11.6.0007.

15. Kulkarni AA, Woeller CF, Thatcher TH, Ramon S, Phipps RP, Sime PJ. Emerging PPARγ-independent role of PPARγ ligands in lung diseases. *PPAR Res.* 2012;2012. Doi:10.1155/2012/705352.

16. Gani OABSM, Sylte I. Molecular recognition of docosahexaenoic acid by peroxisome proliferator-activated receptors and retinoid-X receptor [alpha]. *J Mol Graph Model.* 2008;27(2):217-224. doi:10.1016/j.jmgm.2008.04.008.

17. Bantilan CMK. The effect of postnatal growth restriction on serum fatty acids and lung fatty acid interacting proteins in the rat [master's thesis]. Salt Lake City: University of Utah; 2017

18. Wakabayashi K-I, Okamura M, Tsutsumi S, et al. The peroxisome proliferator-activated receptor/retinoid X receptor heterodimer targets the histone modification enzyme PR-Set7/Setd8 gene and regulates adipogenesis through a

positive feedback loop. *Mol Cell Biol*. 2009;29(13):3544-3555. doi:10.1128/mcb.01856-08.

19. Judge MP, Harel O, Lammi-Keefe CJ. Maternal consumption of a docosahexaenoic acid–containing functional food during pregnancy: benefit for infant performance on problem-solving but not on recognition memory tasks at age 9 mo. *Am J Clin Nutr*. doi:10.1093/ajcn/85.6.1572.

20. Hwang JS, Rehan VK. Recent advances in bronchopulmonary dysplasia: pathophysiology, prevention, and treatment. *Lung.* 2018;(January):1-10. https://doi-org. ezproxy.lib.utah.edu/10.1007/s00408-018-0084-z

21. Itoh T, Murota I, Yoshikai K, Yamada S, Yamamoto K. Synthesis of docosahexaenoic acid derivatives designed as novel PPARγ agonists and antidiabetic agents. *Bioorganic & Medicinal Chemistry*. 2006;14(1):98-108. doi:10.1016/j.bmc.2005.07.074.

22. Collins CT, Makrides M, McPhee AJ, et al. Docosahexaenoic acid and bronchopulmonary dysplasia in preterm infants. *N Engl J Med.* 2017;376:1245-55. doi:10.1056/NEJMoa1611942.

23. Mcgowan SE, Jackson SK, Doro MM, Olson PJ. Peroxisome proliferators alter lipid acquisition and elastin gene expression in neonatal rat lung fibroblasts. *Am J Physiol Lung Cell Mol Physiol*. 1997;273(6). doi:10.1152/ajplung.1997.273.6.l1249.

24. Joss-Moore LA, Wang Y, Yu X, et al. IUGR decreases elastin mRNA expression in the developing rat lung and alters elastin content and lung compliance in the mature rat lung. *Physiol Genomics*. 2011;43(9):499-505. doi:10.1152/physiolgenomics.00183.2010.

25. Sugitani H, Hirano E, Knutsen RH, et al. Alternative splicing and tissue-specific elastin misassembly act as biological modifiers of human elastin gene frameshift mutations associated with dominant cutis laxa. *J Biol Chem*. 2012;287(26):22055-22067. doi:10.1074/jbc.m111.327940.

26. Calder PC. Omega-3 fatty acids and inflammatory processes. *Nutrients*. 2010;2(12):355-374. doi:10.3390/nu2030355.